Biochemistry of Proteins 2.463 Mid-Term Test Feb. 22 1994

Answer all questions in the Exam Booklets. Put your name and student number on all exam booklets.

You may use a calculator and diagrams where appropriate.

The total number of marks is 75 and you have 75 minutes so spend about 1 min per mark *ie* 20 min for a 20 mark question *etc*.

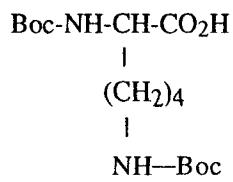
Do *1* of the following 2 questions. Each is worth 20 marks

1 Describe the chemical steps involved in the Edman degradation and explain the main advantages of gas phase sequencing.

2 Explain the separation of molecules by reverse phase HPLC referring to the role and nature of the mobile and stationary phases. What would occur if the silanol beads were not "capped"?

Answer question 3. It is worth 20 marks. a(3) b(7) c(10)

3. a) An aspiring peptide chemist, Uri Thane, has decided to attempt the synthesis of a peptide Gly-Lys-Ala using the solid phase method. To the Ala-resin he couples the following derivative of lysine:



α,ε-di-Boc-lysine

Why has Uri chosen to protect lysine with *two* Boc groups?

b) After the coupling, Uri deprotects the resin-bound peptide with anhydrous CF_3CO_2H and then completes the synthesis in the usual way by coupling Boc-Gly, deprotecting the peptide, and removing it from the resin. Thane is shocked to find a mixture of several peptide products. Two of them give the amino acid analysis (Ala, Gly, Lys) and the third gives (Ala, Gly_2, Lys). Suggest a structure for each product and explain what has happened.

c) Suggest a protected Lysine derivative that could be used to avoid the mixture above, and, assuming the availability of this derivative, outline the steps of a solid-phase peptide synthesis that would yield the final product Gly-Lys-Ala.

Do *1* of the following 2 questions. Each is worth 20 marks.

5 Describe the determination of protein molecular mass by FAB mass spectrometry.

6 Describe, in detail, the results of the attempted solid phase synthesis of Interleukin 3. How was the product characterized and what were the results of the analyses? Explain what is meant by "Preview". What was learned about the protein from its synthesis?

Do all of the following questions. Each is worth 7.5 marks.

7. A 10 micromolar solution of a newly discovered protein Amazin, has an absorption at 280 nm of 0.412 and an absorption at 288 nm of 0.279, in a cell of 1 cm path length. The extinction coefficients (in $l\ mol^{-1}\ cm^{-1}$) for tyrosine and tryptophan at the two wavelengths are listed below. From the information given calculate the number of Tyrosine and Tryptophan residues in Amazin.

	ε_{280}	ε_{288}
Trp	5690	4815
Tyr	1280	385

8. A peptide is synthesized by the solid phase method with a total yield per cycle of 98%. The sequence of the desired product is GAME. What is the nature of the main impurities in the final product and what fractions does each compose of the total mixture?

Bonus Question: Worth 5 points

An expert peptide chemist, A. Mide Bond, has been given the task of synthesizing the following peptide:

Ala-Ile-Val-Leu-Phe-Gly-Phe-Ala-Val-Val-Gly

She elects to use a fragment condensation strategy and synthesize the fragments Boc-Ala-Ile-Val-Leu-Phe-Gly and Phe-Ala-Val-Val-Gly-methyl ester and join them by a mixed anhydride procedure. In view of the racemization problem, why is her choice to join the fragments at Gly a particularly wise one?